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OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Interim Progress Report - Pepcon Superfund Site

FROM: Ken W. Brown, Director, TSC
Characterization and Monitoring Branch, ESD

TO: Kevin Mayer, Superfund Project Manager, SFD 7-2
Region IX

Kevin, for your information and use, a technical review of available analytical data concerning groundwater sample analyses for perchlorate at and near the Pepcon site has been completed. Maps have been obtained showing well locations and the suspected extent and direction of the migrated perchlorate plume from this site. As you are aware, this effort is quite challenging due to the presence of a similar situation at the Kerr-McGee facility located nearby.

The TSC objectives are to address the following topics: (1) Are the migration patterns and plumes separate and distinct for the two sources?; (2) what is the interfering anion, found in some samples, that elutes at a retention time similar to that of perchlorate, and can be rendered non-interfering?; (3) what effect does high total dissolved solids (TDS) (salts; total TDS) have upon the quantitation of perchlorate in these groundwater samples?; (4) what are the chemical compositions of the salts contributing to the TDS and how can these salts be removed, or rendered non-interfering, for the determination of perchlorate?; (5) are the Pepcon and Kerr-McGee plumes distinguishable chemically?

Progress to date has indicated what type of analytical approach, and improvements to quality assurance, are likely to be successful on the high-TDS samples. Samples from monitoring wells have been requested. A meeting with Brenda Pohlman, Remedial Action Program Supervisor, State of Nevada Department of Conservation and Natural Resources, to discuss sample collection, and some additional analysis ,(i.e., as we discussed) is scheduled for December 3, 1998 and I will keep you informed as we progress. Following analysis, interpretations of data will be made to address the above technical issues. Additional details of current progress and plans to complete this project are attached.

Attachment

cc: Mike Gill, RPM, Region IX, SFD-8-B

To address the technical issues noted above, the following approach was applied, with results discussed under each topic:

(a) review recent chemical literature and contact specialists at IC manufacturers to discover what studies have been performed already, and what columns and eluents (e.g. hydroxide vs. carbonate) have been found suitable for perchlorate determination, measurement of TDS, and identification of the anions of interest through retention times on one or several ion chromatographic columns

Chemical Abstracts was searched to the current 1998 issue received by the University of Nevada-Las Vegas library (Vol. 129 #17), for perchlorate related articles of potential interest to this project. Since abstracts of articles may not appear in Chemical Abstracts for some months after publication, the following journals were also individually searched:

Journal	Years	Comments
Analytical Chemistry	1997-8	
Environmental Science & Technology	1997-8	
Journal of Chromatography, Part A	1996-8	checked Vol. 702-821
Chromatographia	1997-8	checked through 9/98
Analusis	1997	1998 out for binding
Analyst	1997-8	
Analytica Chimica Acta	1997-8	checked Vol. 337-374
Talanta	1997-8	checked Vol. 44-45
Fresenius' Journal of Analytical Chemistry	1997-8	checked Vol. 357-360

One recent publication on perchlorates in groundwater was located: D. Herman and W. Frankenberger, Journal of Environmental Quality 27, 750-754 (1998). A copy is attached. Experts at Waters (Jim Krol) and Dionex (Peter Jackson) were contacted for updates on their studies. Howard Okamoto of the State of California-Department of Health Services (Cal-DHS) was also contacted.

These experts feel that the California method is based upon a good approach, and that the Cal/Dionex approach with hydroxide eluent is probably the best one for samples containing high total dissolved solids (TDS). Carbonate eluent, which Waters and others' columns use, is less suitable. It is likely that thiocyanate (SCN-) or thiosulfate (S_2O_3=) is likely the analytical interference for perchlorate that was noted in some samples. Adding a little acetonitrile might help if the membrane can work with this modifier. A complexing metal might also help if the sulfur containing anions would complex with it and perchlorate would not. Loss of efficiency is a possible problem with this approach.

It appears likely that thiosulfate or another sulfur-containing anion (possibly an impurity in technical-grade thiosulfate) is more likely to be the interference than thiocyanate in water samples. Cutting the injection volume down from the 1 mL used by the California protocol to achieve the low ppb detection limit is an approach that could improve the ability of the column to handle the TDS. Either 100 μL or 50 μL sample loops could be used to improve performance on samples with high TDS. These injection volumes could still give adequate detection limits with these samples that presumably have perchlorate levels much higher than low-ppb levels for which the 1 mL injection volume was needed. The ion chromatograph could be recalibrated with logical standards for these revisions to the method. These improvements to quality assurance could be incorporated into the method and into QA Plans, pending laboratory verification of their effectiveness.

A barium cartridge could be used to remove sulfate, and standards could be used to verify that no perchlorate is removed. It is not easy to remove or separate thiosulfate. The AS-16 column could be used to achieve separation similar to that with the AS-11. The AS-16 column also has higher sample capacity than the AS-11. The AS-16 can use a weaker eluent and gradient elution to optimize separation of analytical interferences to perchlorate. For example, with 35 mM hydroxide eluent, the retention time for perchlorate is 19 minutes, and thiosulfate elutes at 6 minutes. Both Alltech and Dionex offer disposable cartridges designed to remove anions such as chloride, sulfate, and bicarbonate/carbonate. Using such cartridges would be expected to add to the cost of analysis, and would require additional QA/QC to ensure that perchlorate is not lost during the extra sample preparation steps.

The Department of Health of Utah is reportedly running samples with 60-70 mM hydroxide and achieving 15-16 minute retention times for perchlorate. Weakening the eluent strength lengthens retention times, and may degrade the detection limit, but improves the separation of perchlorate from interferences.

(b) contact State of Nevada and State of California personnel to determine the status of their studies on these issues, and identify further straightforward analyses that should help resolve these analytical issues

Brenda Pohlmann of the Nevada Division of Environmental Protection (NV-DEP) provided maps of the Pepcon site area, including the groundwater monitoring well locations. Analytical data were also provided for review.

Some samples from the area between the mapped locations for the two groundwater plumes were reported "non-detect" for perchlorate. Examination of the chromatograms (copies are attached) for the "French Drain" and "Well PC18" revealed that two closely-eluting but distinguishable peaks were present, in the retention time range for perchlorate anion. These samples were #L9805040-01 and -02 (980507128, 980507129). The non-detect reports were therefore not indicative of absence of perchlorate, but rather of method deficiency for these difficult samples. These samples also contained high TDS. In general, it was found that when the TDS was high, over about 18,000 μS, the California protocol became unreliable. In defense of this method, however, it should be noted that it was intended primarily for drinking water or other relative clean waters and for detection limits in the low ppb range. The medium-TDS samples (ca. 10,000 μS) in which perchlorate was reported were found to contain high concentrations of perchlorate (several hundred ppm). They could be diluted, or smaller amounts could be injected into the ion chromatograph to facilitate detection of perchlorate in the presence of high TDS and potential analytical interferences.

Howard Okamoto of Cal-DHS provided input and a list of laboratories. A copy of his memo is attached.

(c) complete a data review on available data to identify, and list for further testing, the existing groundwater monitoring wells that contain high TDS and/or the anion that coelutes with perchlorate

A review of available data resulted in locating the following samples having both high TDS and results reported as non-detect (ND). It is quite possible that perchlorate was present in these samples, and perhaps at high concentrations. These samples would be good candidates for further study.

Sample Set	Sample number (suffix)	conductivity, μS/cm
L9805126	-23	53,100
L9805106	-07	20,200
	-08	19,000
	-09	18,000
	-12	20,500
	-14	21,400
L9805173	-01	34,000
L9805040	-01	17,700
	-02	14,200

(d) request groundwater monitoring samples from these locations for analysis

American Pacific Corporation plans to sample some monitoring wells in the near future, and we provided input to Ms. Pohlmann regarding which wells would be expected to yield useful samples (attached is a copy of the letter from American Pacific with our list of the desired wells handwritten next to their typed list of proposed wells). Such samples would include those from the geographical area near and between the expected Pepcon and Kerr McGee groundwater plumes, having high TDS and/or an analytical interference for perchlorate analysis.

Following receipt of these samples we plan to continue the approach listed in the letter of October 16: (e) obtain routine-type analyses through a contractual mechanism to an outside laboratory on these samples, to include perchlorate determination, measurement of TDS, and identification of the anions of interest through retention times on one or several ion chromatographic columns; (f) review new and prior analytical data to assess the migration patterns of the two plumes (Pepcon vs. Kerr-McGee) so that a conclusion can be made regarding whether the plumes are separate or converging; (g) assess the status of perchlorate determination in the presence of high TDS before and after the application of information gathered in this study; (h) obtain routine full-suite analyses of Pepcon and Kerr-McGee groundwater in the suspected plume areas to determine whether there is some unique ion or other analyte (e.g. organic) present in the former source to select an indicator parameter for determination of whether that plume migrates into the Kerr-McGee plume.

Once we are informed by NV-DEP about the time frame for sample collection by American Pacific, we will contact the contract laboratories listed by Cal-DHS and request their technical approaches and per-sample costs regarding the analysis of these high-TDS samples containing potential analytical interferences for perchlorate.

We will evaluate their technical responses and costs-per-sample proposals to determine which laboratory or laboratories are most likely to provide satisfactory analytical results and point the way for possible method improvements to obtain satisfactory analyses on such samples in the future. It is expected that a successful, rugged, and economical approach for high-TDS samples will combine the AS-11 (or better, AS-16) column with lower injection volumes and/or lower eluent strength, and possibly supplemented by pre-treatment with a disposable column to remove bulk anions. We anticipate that one or two laboratories will analyze some or all samples, depending on cost and our assessments of their technical approaches.

Results of these analyses will be used to develop quality assurance improvements for the analysis of high-TDS samples for perchlorate.

REVIEWS AND ANALYSES

Microbial-Mediated Reduction of Perchlorate in Groundwater

David C. Herman and William T. Frankenberger, Jr.*

ABSTRACT

Perchlorate has been widely used as a propellant in solid rocket fuel, and has recently been identified as a contaminant in both groundwater and surface waters. Perchlorate is recognized by the U.S. Environmental Protection Agency (USEPA) as a potential health risk, and the State of California has set a drinking water action level of 18 $\mu g\ L^{-1}$. Incidents of groundwater contamination have been associated with industrial sites in California and Nevada that have been involved in the manufacturing or testing of solid rocket propellants. Microorganisms have been shown to be capable of reducing perchlorate (ClO_4^-) to chloride (Cl^-) and oxygen, thus transforming perchlorate into innocuous end-products. Bioreactor processes for the remediation of perchlorate contaminated wastewater have previously been established. However, these systems were optimized for perchlorate concentrations in the grams per liter range, while groundwater contamination can be a million-fold lower but still exceed the water quality action level. This literature review will focus on microbial-mediated perchlorate reduction, and discuss issues of importance to the remediation of perchlorate-contaminated groundwater.

Perchlorate (ClO_4^-) is an oxyanion that has been used extensively in the chemical and aerospace industries because it can act as a strong oxidizing agent. The mishandling of perchlorate at aerospace-related industrial sites is the likely source of perchlorate that has recently been discovered in surface and groundwaters. The persistence of perchlorate in the environment and its toxicity to humans at sufficient concentrations has raised concern over drinking water quality and possible environmental impacts. Perchlorate is not currently regulated under the Safe Drinking Water Act, although the California Department of Health Services has established an action level for perchlorate in drinking water of 18 $\mu g\ L^{-1}$. The basis of the action level is an evaluation of toxicity data by the USEPA (details are available from the California Department of Health Services homepage at www.dhs.cahwnet.gov). Perchlorate has been found in certain drinking water wells in California at concentrations that exceed the action level. In response, the California Department of Health Services has advised that water from these wells should not be used as a source of drinking water.

The development of effective and efficient strategies for the remediation of perchlorate in groundwater is an area of intense interest. Remediation strategies for the removal of perchlorate based on adsorption by activated C have not proven to be effective due to rapid saturation of perchlorate adsorption sites. Other advanced procedures for the removal of perchlorate include reverse osmosis and ion exchange, which are very expensive. An alternative strategy is the reduction of perchlorate by a biological means. Reduction of perchlorate provides an attractive remediation strategy because complete reduction can transform the compound into innocuous end-products, namely, chloride and oxygen. Biological-mediated reduction has been used successfully in the remediation of other toxic compounds, including chromate Cr(VI) (Losi et al., 1994a, b), selenate and selenite (SeO_4^{2-} and SeO_3^{2-}) (Losi and Frankenberger, 1997), as well as in the removal of nitrate (NO_3) and nitrite (NO_2) from treated wastewater (Matějů et al., 1992). This review will evaluate microbial-mediated perchlorate reduction as a biological remediation strategy.

PERCHLORATE IN THE ENVIRONMENT

The primary use of perchlorates is in solid rocket fuel. Solid rocket propellants can contain as much as 70% ammonium (NH_4) perchlorate as finely ground, crystalline particles dispersed within a polymer matrix. The polymer-binding matrix, with added aluminum, act as the fuel that reacts with the oxidizer. Ammonium perchlorate is also used in the production of explosives, pyrotechnics, and blasting formulations. Other perchlorate forms are used in dry batteries and oxygen-generating systems. The U.S. domestic capacity of NH_4 perchlorate is estimated to be more than 30 000 t /yr, with actual production depending on demand (Kirk-Othmer Encyclopedia of Chemical Technology, 1996). Wastewater generated from the manufacturing, maintenance, and testing of solid rocket propellants can contain NH_4 perchlorate concentrations in the grams per liter range.

The problem of perchlorate contamination of water supplies is a recently emerging issue. Much of the information presented below concerning the occurrence of perchlorate contamination is accessible from the California Department of Health Services home page at www.dhs.cahwnet.gov. There are several case histories of perchlorate in groundwater at industrial sites operated by aerospace companies. The Aerojet General Corporation, near Sacramento, CA, operated a facility primarily involved in the development and testing of rocket fuels. Aerojet has been treating water from a shallow aquifer to remove volatile organic chemicals, such as TCE, and has found this water also contains approximately 8 $mg\ L^{-1}$ perchlorate. Aerojet is in the development stages of testing a granulated active C/fluidized bed reactor system to remove perchlorate from the water. In Nevada, sampling of wells at a current and former site of NH_4 perchlorate manufacturing were reported to have perchlorate concentrations >600 $mg\ L^{-1}$ (Las Vegas Sun, see homepage at www.lasvegassun.com).

Perchlorate contamination has been shown to be a wide-

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Abbreviations: BOD, biochemical oxygen demand.

spread problem. A survey reported by the California Department of Health Services revealed that of 53 wells tested in northern California, and 449 wells tested in central and southern California, perchlorate levels exceeded the 18 $\mu g\ L^{-1}$ action level in 8 and 25 wells, respectively. Testing of drinking water wells in Riverside and San Bernardino Counties (California) revealed some wells to contain as much as 216 $\mu g\ L^{-1}$ perchlorate, resulting in the closure of nine wells. Major surface water systems have also been shown to contain perchlorate, as indicated by levels as high 165 $\mu g\ L^{-1}$ found in some areas of Lake Mead (Colorado River) and 8 $\mu g\ L^{-1}$ in river water south of the lake.

TOXICITY OF PERCHLORATE

Medical knowledge concerning perchlorate toxicity stems from its use as an antithyroid agent in the treatment of Graves' disease. Graves' disease is a common cause of hyperthyroidism with a prevalence estimated to be one to two cases per 1000 yr^{-1} (Wilson and Foster, 1992). Perchlorate had been used in the treatment of hyperthyroidism because it can act to reduce thyroid iodide transport, thus decreasing the production of thyroid hormones. Due to its potential toxicity, perchlorate has been replaced by superior drug treatments. There is little information concerning the toxicity of perchlorate to humans. Adverse effects on the thyroid have been reported at doses that would correspond to drinking water concentrations of 49 mg L^{-1} (see California Department of Health Services homepage for more details). Based on available toxicity data, a water quality action level has been set at 18 $\mu g\ L^{-1}$ by California's Department of Health Services. The health effects of long-term, low-level exposure are currently being investigated.

OTHER CHLOROXYANIONS

There is more information available concerning the microbial reduction of chlorate (ClO_3^-) than of perchlorate. The major use of sodium chlorate is in the generation of chlorine dioxide for bleaching of wood pulp. Chlorine dioxide cannot be transported and is therefore generated on site by pulp producers. Chemical bleaching alone was estimated to use 921 000 t yr^{-1} in 1990 (Encyclopedia of Chemical Technology, 1996), which is 30 times greater than perchlorate production. Some of the chlorine dioxide used in the bleaching process is released as chlorate, and it has been reported that chlorate concentrations in pulp mill effluents can be as high as 53 mg L^{-1} (Rosemarin et al., 1994). Chlorates are also used in the manufacturing of perchlorates, and as defoliants and desiccants in the production of cotton (*Gossypium herbaceum*) and soybean (*Glycine max* L.), respectively. Chlorates can also be released into the environment from the use of chlorine dioxide and hypochlorite as disinfectants in water treatment.

The environmental impact of chlorate was reviewed by van Wijk and Hutchinson (1995) who reported that most aquatic organisms can tolerate high concentrations of chlorate (>100 mg L^{-1}). The exception is the macro brown algae that were sensitive to 0.1 mg L^{-1} chlorate. The toxicity of chlorate in pulp mill effluents to macro brown algae (e.g., *Fucus* spp.) in the Baltic Sea has been investigated (Lehtinen et al., 1988; Rosemarin et al., 1994). The mechanism of toxicity is not completely understood although it is believed that chlorate itself is not toxic, but rather it is transformed into a more toxic metabolite, chlorite (ClO_2^-). Many plants, algae, and bacteria can transform chlorate to chlorite by assimilative and dissimilative NO_3^- reduction enzyme systems. Two features of the NO_3^--reductase enzyme system are important in terms of chlorate toxicity. Firstly, NO_3^- reductase is an inducible enzyme, and secondly, the NO_3^--reductase enzymes in certain species have a much higher affinity for NO_3^- than chlorate (van Wijk and Hutchinson, 1995; Balch, 1987). Therefore, NO_3^- concentration is a modulator of chlorate toxicity. In environments where NO_3^- is limiting, or when chlorate levels are much greater than NO_3^- levels, the toxicity of chlorate can be enhanced.

Although the transformation of chlorate into chlorite is thought to be a key factor in chlorate toxicity, the difference between sensitive and resistant organisms remains unexplained. It was hypothesized by van Wijk and Hutchinson (1995) that nonsensitive species are capable of reducing chlorate all the way to chloride, without the formation of a toxic intermediate.

The activation of chlorate toxicity has been used in the selection of NO_3^- reductase-deficient bacteria from anaerobic cultures. The mutants are resistant to chlorate because they lack the NO_3^- reductase that activates chlorate through its reduction to chlorite. However, other bacteria have shown the capability to use chlorate as a final electron acceptor during the anaerobic respiration of organic compounds (as will be discussed in more detail in a later section). The complete reduction of chlorate to chloride and oxygen under anaerobic growth conditions represents a biotreatment strategy that has been successfully applied to the remediation of effluent produced by the pulp and paper industry (Malmqvist et al., 1991; Malmqvist and Welander, 1994).

MICROBIAL-MEDIATED REDUCTION OF PERCHLORATE

Microbial-mediated reduction of perchlorate can be viewed as a promising strategy for the remediation of contaminated water. Microbial reduction of oxyanions can occur as the result of anaerobic respiration. Microbial respiration couples the oxidation of an organic substrate, such as glucose or acetate, to the reduction of a final electron acceptor, usually oxygen. Under anaerobic conditions, the oxidation of organic compounds requires the use of an alternative electron acceptor in place of oxygen, such as NO_3^-, Mn(IV), Fe(III), or SO_4^{2-}. Bacteria capable of anaerobic respiration are common to soil and sediments where anaerobic conditions are prevalent and natural sources of alternate electron acceptors are common. As a highly oxidized compound (+7 oxidation state), perchlorate has a high potential for utilization as an alternate electron acceptor. Rikken et al. (1996) provided equations for the stoichiometric reaction of acetate with oxygen and perchlorate as electron acceptors.

$$CH_3COO^- + 2O_2 \rightarrow 2\ HCO_3^- + H^+$$

$$\Delta G^{o\prime} = -844$$

$$CH_3COO^- + ClO_4^- \rightarrow 2\ HCO_3^- + H^+ + Cl^-$$

$$\Delta G^{o\prime} = -966$$

Comparing the Gibbs free-energy changes ($\Delta G^{o\prime}$, KJ mol acetate^{-1}) it is evident that perchlorate reduction is energetically favorable.

There are several reports of mixed bacterial cultures capable of reducing perchlorate under anaerobic growth conditions. These bacteria were enriched from samples of sewage sludge in media containing various sources of organic C, mineral nutrients, and high concentrations of perchlorate (500–1000 mg L^{-1}, depending on the study). Acetate was shown to serve as the sole C source for the reduction of (per)chlorate (Rikken et al., 1996; van Ginkel et al., 1995; Malmqvist et al.,

1994; Korenkov et al., 1976). Using an acetate medium, Rikken et al. (1996) reported that 800 mg L^{-1} perchlorate was completely reduced after 9 d. In contrast, Attaway and Smith (1993) used a mixture of nutrient broth and yeast extract for the enrichment of a perchlorate-reducing culture, and reported reduction of 1000 mg L^{-1} perchlorate after approximately 2 d. They determined that perchlorate reduction occurred between pH 6.6 and 7.5, with an optimum at 7.1, and a temperature range between 25 to 42°C, with an optimum at 42°C.

Perchlorate-reducing bacteria include *Vibrio dechloraticus* Cuznesove B-1168 (Korenkov et al., 1976; Romaneko et al., 1976), *Wolinella succinogenes* HAP-1 (Wallace et al., 1996), and a proteobacteria, strain GR-1, described by Rikken et al. (1996). Bacteria capable of reducing chlorate include *Ideonella dechloratans* (Malmqvist et al., 1994) and an *Acinetobacter* sp. (Stepanyuk et al., 1992).

The reduction of (per)chlorate under anaerobic growth conditions has been shown to be directly proportional to the release of chloride. This would indicate that perchlorate can be completely reduced to chloride and oxygen (Rikken et al., 1996; van Ginkel et al., 1995; Attaway and Smith, 1993; Malmqvist et al., 1991). The reduction of (per)chlorate was also accompanied by biomass production, indicating that the microbial reduction of (per)chlorate is coupled to energy-yielding reactions (Rikken et al., 1996; Malmqvist et al., 1991). Rikken et al. (1996) proposed the following pathway for the reduction of perchlorate.

$$\underset{\text{perchlorate}}{ClO_4^-} \rightarrow \underset{\text{chlorate}}{ClO_3^-} \rightarrow \underset{\text{chlorite}}{ClO_2^-} \rightarrow \underset{\text{chloride}}{Cl^-} + O_2$$

They reported that anaerobic oxidation of an organic C source (acetate) was coupled to the reduction of perchlorate to chlorate and of chlorate to chlorite. However, the bacterium did not derive physiological useful energy from the reduction of chlorite to chloride. Chlorite can be inhibitory to microbial activity, and the transformation of chlorite to chloride is believed to be an enzymatic detoxification mechanism that protects the cell and allows the bacterium to use perchlorate and chlorate as electron acceptors. The purification and characterization of the chlorite-reducing enzyme has been reported (Wallace et al., 1995; van Ginkel et al., 1996). A perchlorate-reducing proteobacteria was found to contain a heme iron enzyme that catalyzed chlorite to chloride and oxygen (van Ginkel et al., 1996). The enzyme, chlorite dismutase, displayed maximal activity at pH 6.0 and 30°C, and was also found to obey Michaelis-Menton kinetics. The calculated kinetic parameters, V_{max} of 2200 U mg^{-1} (protein), where one unit (U) of activity is the amount of enzyme required to convert 1 μmol of chlorite/min, and K_{max} of 170 μM, indicated that the dismutase is efficient in removing chlorite. Strain *W. succinogenes* HAP-1 demonstrates a chlorite dismutase activity that is at least 1000-fold greater than the perchlorate or chlorate-reductase activities. Therefore, the chlorite produced during (per)-chlorate reduction should not accumulate to toxic levels (William Wallace, 1998, personal communication).

The (per)chlorate-reducing bacteria currently described are capable of using NO_3^- as an electron acceptor in anaerobic respiration. It has been suggested that the enzymatic activity supporting the reduction of chloroxyanions is linked to the enzymes involved in NO_3^- reduction, namely, NO_3^- reductases (Romaneko et al., 1976). However, not all denitrifying bacteria are capable of growth with chlorate as an electron acceptor, and there is evidence for a specific (per)chlorate reductase enzyme system that is separate from NO_3^- reduction. In the case of *W. succinogenes* HAP-1 (Wallace et al., 1996), the presence of NO_3^- did not interfere with perchlorate reduction. This would suggest that the enzymes involved in perchlorate reduction were not necessarily the same as those involved in NO_3^- reduction. The chlorate-reducing isolate, *I. dechloratans*, described by Malmqvist et al. (1994), lost its ability to reduce NO_3^- when grown for long periods on chlorate as the sole electron acceptor. Malmqvist et al. (1994) suggested that chlorate reduction is performed by a modified NO_3^--reducing enzyme system, and that this could explain the occurrence of chlorate reducers even though chlorate has been present in the environment only as a result of human activity and only for a short period, from an evolutionary perspective, for the development of an enzyme system for the utilization of chlorate as an electron acceptor in anaerobic respiration.

Oxygen is a major inhibitor of (per)chlorate reduction (van Ginkel et al., 1995), and exposure of an active perchlorate-reducing culture to air can immediately halt perchlorate reduction (Attaway and Smith, 1993). Attaway and Smith (1993) also noted that cultures which were actively reducing perchlorate could reach a stage in which perchlorate reduction ceased, and the medium would be in an oxidized state, as indicated by a change in color of a redox indicator, resazurin, from clear to pink. They reported that the oxidation of the medium was probably caused by the formation of transient chloride oxide metabolites, possibly chlorite or hypochlorite. Perchlorate reduction would resume with the addition of a reducing agent, cysteine hydrochloride, which reduced the resazurin from pink to clear. The need to maintain redox conditions in which resazurin is converted from pink to clear to sustain perchlorate reduction would indicate a redox potential requirement of less than −110 mV for perchlorate reduction.

The choice of a C substrate to act as an electron donor can be an important consideration. Acetate has been used successfully to support (per)chlorate reduction (Rikken et al., 1996; van Ginkel et al., 1995; Malmqvist et al., 1994; Korenkov et al., 1976). In contrast, Attaway and Smith (1993) enriched perchlorate-reducing bacteria using a nutrient broth/yeast extract mixture, and then tested a variety of C substrates to determine their suitability for support of perchlorate reduction. They noted that a wide variety of organic acids, including acetate and alcohols promoted growth without sustaining perchlorate reduction. Perchlorate reduction was only evident with the addition of protein-based C sources, including nutrient broth, peptone, yeast, and casamino acids. In a study using a chlorate-reducing mixed culture that had been enriched with acetate, van Ginkel et al. (1995) noted that a wide variety of organic substrates, including alcohols, carboxylic acids, and amino acids could be oxidized to support chlorate reduction. Fermentable substrates, such as glucose, lactose, carboxylmethyl cellulose, and starch, did not directly support chlorate reduction, although it was shown with glucose that the formation of fermentation products, acetic acid and formic acid, supported chlorate reduction.

BIOREACTOR SYSTEMS FOR PERCHLORATE REDUCTION

Several patents have described processes for the use of microbial-mediated reduction of perchlorate as a means to remediate industrial waste. A Yakovlev et al. (1973) patent describes the use of unaerated sewage sludge for the treatment of certain oxygen-containing inorganic chlorine and metal compounds, including perchlorate, chlorate, and chromate. Domestic sewage sludge was mixed with contaminated wastewater and placed in a large tank. In the absence of aeration, microbial utilization of organic material within the sludge rapidly depleted the available oxygen. Under anaerobic condi-

tions, the reduction of oxygen-containing inorganic compounds occurred with the oxidation of organic compounds. Following the anaerobic phase, a second stage in the process removed the sludge from the water by precipitation. It is important in this process to supply an excess quantity of organic material, as measured by biochemical oxygen demand (BOD), to ensure the creation of an anaerobic environment.

Several later patents have improved on this basic process, enhancing the rate and extent of perchlorate reduction, and enabling the treatment of higher concentrations of perchlorate in wastewater. Korenkov (1976) described a method of reducing perchlorate and chlorate under anaerobic conditions using the bacterium, *V. dechloraticans* Cuznesove B-1168. This organism is capable of reducing perchlorate and chlorates when grown anaerobically on acetate or ethanol as a C source (Romanenko et al., 1976). The authors reported reduction rates of perchlorate as high as 70 mg ClO_4^- h^{-1} g^{-1} biomass solids (dry wt.), and the ability to treat perchlorate concentrations as high as 3 m*M* (about 300 mg L^{-1}). A 1994 patent (Attaway et al., 1994) describes a process in which contaminated water is added to an anaerobic bioreactor and spiked with a mixed bacterial culture. The bacterial culture contains a specific bacterium, *W. succinogenes*, which was isolated from domestic sewage sludge for its ability to reduce very high concentrations (>7000 mg L^{-1}) of perchlorate (Attaway and Smith, 1993; Wallace et al., 1996). High protein organic nutrients were found to support perchlorate reduction, and the source of this oxidizable organic matter in the anaerobic bioreactor could be in the form of aged brewers yeast, cottonseed protein or whey powder. A second stage in the process removes nutrients and organic matter to improve the quality of the water for discharge. One advantage of this system is that it does not use sewage sludge, and therefore eliminates problems associated with the presence of pathogens. The bacterium isolated was capable of reducing perchlorate concentrations 26-fold greater than in previous reports, and was reported to have a specific perchlorate degradation rate of at least 1492 mg ClO_4^- h^{-1} g^{-1} biomass (dry wt.). Through the use of a specific isolate and by optimizing nutrient and environment conditions, the anaerobic reactor was capable of greater perchlorate reduction rates than previously reported.

POTENTIAL USE OF MICROBIA-MEDIATED REDUCTION IN THE REMEDIATION OF PERCHLORATE IN GROUNDWATER

The processes described above dealt with the remediation of perchlorate in the milligram per liter concentrations that are associated with wastewater generated from handling of rocket propellant in an industrial setting. Rikken et al. (1996), van Ginkel et al. (1995), and Attaway and Smith (1993) reported the stoichiometric conversion of perchlorate to chloride, indicating that complete reduction of perchlorate was occurring. However, the limits of detection of the analyses made were not stated. There have been no reports of biotreatment systems optimized for the reduction of perchlorate concentrations in the microgram per liter range; the level of contamination of current concern.

To be effective, a remediation system must ensure the reduction of perchlorate concentrations to less than the current action level of 18 μg L^{-1} and the complete reduction of perchlorate to chloride and oxygen. The requirements for perchlorate reduction includes anaerobic conditions, neutral pH, moderate to high temperatures, and a C source suitable for respiration. However, little attention has been given to the factors that may interfere with perchlorate reduction in a groundwater matrix.

One issue of concern is the presence of a variety of possible electron acceptors in groundwater. In the environment there can be a distinct sequence by which electron acceptors are used (see Table 1). The basis of this sequence is the "selection" by the microbial community of an electron acceptor that will maximize energy yield. A higher energy yield from the oxidation of a C substrate will bestow a competitive advantage to a particular population, such as NO_3^- reducers over sulfate reducers, until the available NO_3^- has been exhausted. The position of perchlorate in the sequence of dominant terminal electron-acceptors is not known.

Bacterial isolates capable of growth via (per)chlorate reduction have been shown to reduce NO_3^- (Attaway and Smith, 1993; Malmqvist et al., 1994; Rikken et al., 1996), manganese [Mn(IV)] (Rikken et al., 1996), and sulfate (Attaway and Smith, 1993). There have been limited and conflicting reports on the ability of these alternative electron acceptors to affect the rate of (per)chlorate reduction. Van Ginkel et al. (1995) found that the presence of NO_3^- competitively inhibited chlorate reduction, while Mn(IV), Fe(III), and sulfate had no influence. In contrast, Wallace et al. (1996) reported that *W. succinogenes* HAP-1 preferentially reduced perchlorate prior to reducing NO_3^-. Attaway and Smith (1993) also showed that the presence of NO_3^- and sulfate did not inhibit the rate of perchlorate reduction. However, they did find that the presence of chlorate competitively inhibited the reduction of perchlorate, and that complete inhibition of perchlorate reduction was evident in the presence of NO_3^- and chlorite, probably as a result of direct toxicity to the cell.

A second consideration that has not previously been addressed is the determination of a threshold concentration of perchlorate that will promote and sustain the growth of perchlorate reducers. Perchlorate concentrations may be too low to support the activity of perchlorate-reducers, and, therefore, not maintain a viable population. This condition may be exacerbated if NO_3^- levels are high relative to perchlorate levels, and the perchlorate reducers are in competition with denitrifiers for nutrients. Such a situation may create problems in the maintenance of a high density of perchlorate-reducing cells. One possible solution would be to pretreat the groundwater to remove NO_3^-. A second possible solution is to supplement the groundwater via chemical analog enrichment to support perchlorate reduction and maintain a perchlorate-reducing population that is competitive with denitrifiers. However, it will be necessary to ensure that chemical analog supplementation will promote the complete removal of all chloroxyanions.

Current efforts to remove selenium (Se) from agricultural drainage waters (Thompson-Eagle and Frankenberger, 1992) can provide valuable insights for the development of a perchlorate bioremediation strategy. Selenium is a trace metalloid found in the Central Valley of California with naturally Se-enriched soils. Intensive agriculture has resulted in the mobilization of Se into agricultural drainage. Excess Se in evapora-

Table 1. The sequence of utilization of electron acceptors most commonly found in groundwater (from Smith [1997] with permission).

Oxidized form	Reduced form	Concentration range generally found in groundwater
O_2	H_2O	0–0.4 m*M*
NO_3^-	N_2	0–20 m*M*
Mn^{4+}	Mn^{2+}	Very low
Fe^{3+}	Fe^{2+}	Very low
SO_4^{2-}	S^{2-}	0–15 m*M*
CO_2	CH_4	0–4 m*M*

tion ponds is toxic to wildlife, and can cause reproductive deformities. The common forms of Se in agricultural drainage are the oxyanions, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). Microbial-mediated reduction has the potential to remove selenium oxyanions in the form of elemental Se, which is a much less soluble and bioavailable form of Se. The effect of NO_3^- on selenate reduction has been an area of intensive study. Oremland et al. (1990) investigated the reduction of NO_3^-, selenate, and sulfate in evaporation pond sediment and found that selenate reduction primarily occurs near the surface of the sediment, above the zone of sulfate reduction, and that selenate is reduced in the same sediment zone as NO_3^-. However, not all NO_3^--reducing bacteria can reduce selenate, and selenate reduction can be inhibited until all the available NO_3^- has been reduced (Steinberg and Oremland, 1990; Steinberg et al., 1992). Several different selenate-reducing bacteria have been isolated from Se-contaminated environments (DeMoll-Decker and Macy, 1993; Losi and Frankenberger, 1997), and have been shown to reduce selenate and NO_3^- simultaneously, and to reduce selenate all the way to elemental Se. In the case of *Thauera selenatis*, which can reduce selenate to selenite, the complete reduction of selenate to elemental Se is dependent on the presence of NO_3^- (DeMoll-Decker and Macy, 1993). The authors concluded that NO_3^- reductase, or a component of the NO_3^- respiratory system, is involved in the reduction of selenite to elemental Se while also reducing NO_3^-. Macy et al. (1993) optimized a biological reactor system for the anaerobic treatment of selenate-NO_3^- containing agricultural drainage water, and, with the addition of *T. selenatis*, reduced 350 to 450 μg L^{-1} Se to 5.4 to 3.6 μg L^{-1} Se.

The Se experience provides incentive for the isolation and characterization of perchlorate-reducing bacteria, and to investigate their ability to reduce perchlorate concentrations to the low microgram per liter range. More research is needed on specific issues related to perchlorate reduction, including the physiological capabilities of perchlorate-reducing isolates, the mechanism of perchlorate reduction, the selectivity of the isolate for perchlorate, the sequence of preference shown by bacterial populations for electron acceptors relative to perchlorate, and the threshold concentration of perchlorate that will maintain and promote the activity of the perchlorate-reducing bacterial population.

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Sample Name : 980507128_1/2
Date Time Collected : 5/14/98 11:16:29 AM
Date Time Updated : 5/14/98 11:30:59 AM
Calibration Date : 5/14/98 7:36:52 AM
Injection Number : 22
System Operator : EYW

System Name : IC_#2
Column Type :
Detector Name :
Module ID : 00 08 ef
Moduleware Version : 02.30

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Method File Name : c:\peaknet\method\ic_#2\clo4as11.met
Dilution Factor : 2.00

Peak Information : All Peaks

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
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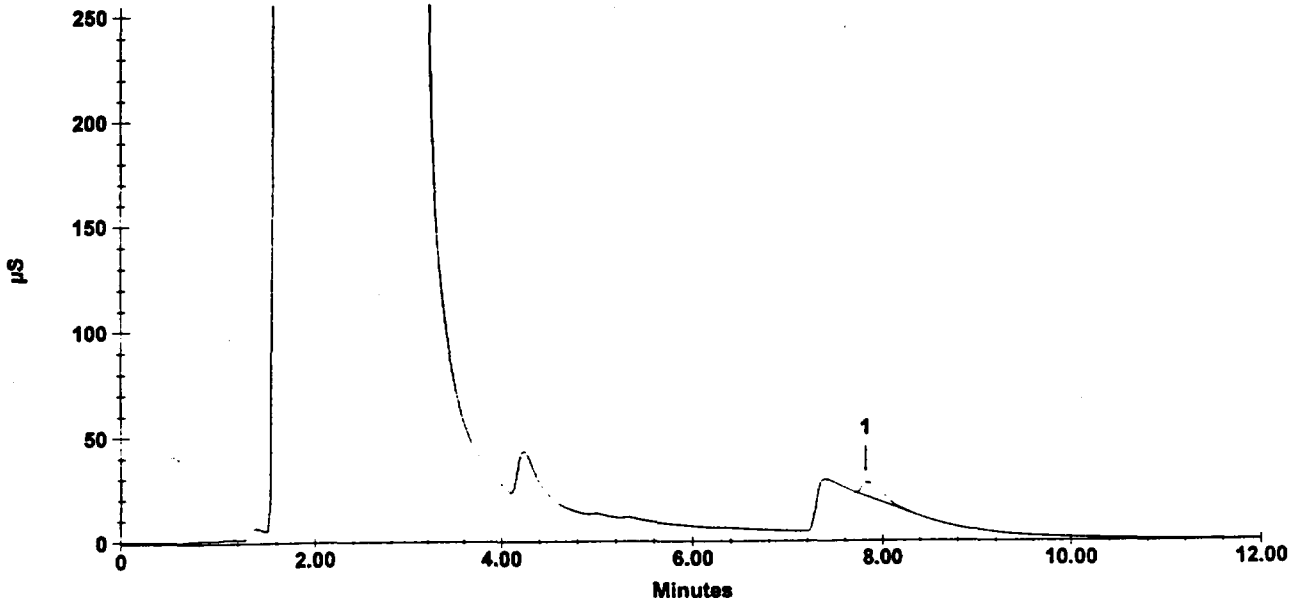
		---total(s)---					
	7.82		0.00	64104	1067929		

980507128_1/2



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System Name : IC_#2
Column Type :
Detector Name :
Module ID : 00 08 ef
Moduleware Version : 02.30

Data File Name : C:\PEAKNET\DATA\MAY98\0513CLO4_022.DXD
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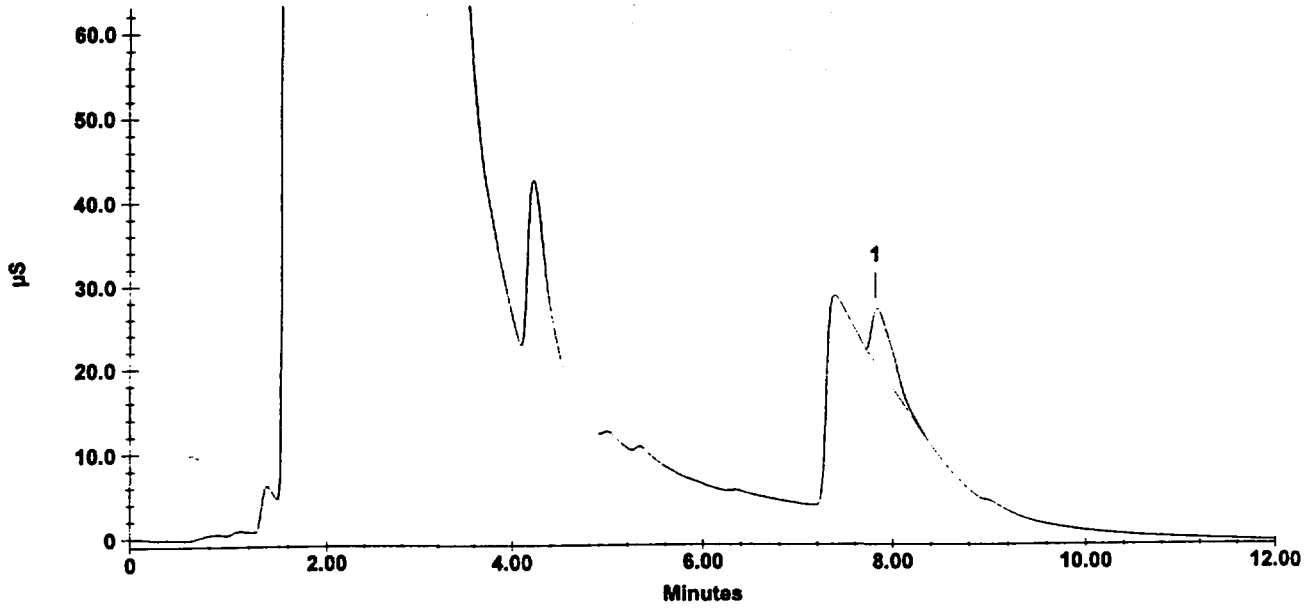
Dilution Factor : 2.00

Peak Information : All Peaks

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	7.82		0.00	64104	1067929	1	
		---total(s)---					
	7.82		0.00	64104	1067929		

980507128_1/2



FAX TRANSMITTAL

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Date: 11/17/98

To: Joe Donnally

Company/Agency: Lockheed Martin Env. Services

Las Vegas, NV

Phone: 702-897-3387

FAX: 702-897-6640

From: Howard Okamoto

Phone: 510-540-2205

Number of pages including cover sheet: 5

Notes/Comments:

Please see the attached list of commercial laboratories in CA approved by the CA DHS to perform perchlorate analysis of drinking water. All of these laboratories have demonstrated their capabilities to perform low-level perchlorate analysis of water. Montgomery-Watson (Pasadena) and Weck Laboratories (City of Industry) are two

laboratories that may have the resources/skills to deal with difficult samples (e.g. high TDS, coeluting interference). You may want to contact each of these two labs to determine what approach each would take to analyze these difficult samples and their associated analytical costs. The other labs on the list may also have the necessary qualifications that you are looking for and it should be worth your time to contact them.

High TDS samples are best analyzed on a column with high capacity. The Dionex AS11 has twice the column capacity (45 µeq) of the AS5 (20 µeq) and should therefore be capable of chromatographing samples with higher TDS than the AS5. Our laboratory tests have demonstrated that the AS5 is capable of chromatographing 5 ppb of perchlorate in reagent water containing 1000 ppm of sulfate, chloride or carbonate without dilution (740 µL sample loop) and with >90% recoveries of the perchlorate. Assuming that the AS11 will be used, it is important not to use an eluent composition >100 mM NaOH, or an eluent flow rate >1 mL/min because too short a retention for perchlorate will result in it being masked by the high concentration of early eluting anions. For high TDS samples, it may even be helpful to increase the perchlorate retention time to better separate it from the early eluting anions (50 mM NaOH may work). The suppressor and detector should obviously be in optimum working condition for proper sensitivity and low baseline noise. Frequent blank analysis, or longer run times may also be required for column cleanout and baseline stabilization.

To determine potential bias in sample results, the samples should be fortied with perchlorate to assess matrix effects on quantitation. The fortification level should be based on the sampling/project objectives. Fortification should also be used whenever there is any question on "identifying" a perchlorate peak, or when a coeluting peak causes uncertainty in "identification."

As a last resort, the high TDS samples may be treated to remove chloride, sulfate and bicarbonate/carbonate, although treatment may add to the cost of analysis. Disposable cartridges that remove these anions are commercially available from Dionex and Alltech. If cleanup is employed, it is important that method blanks, fortified blanks, and fortified samples be included with the samples to be treated in order to monitor for potential biases contributed by the treatment procedures. The quality control samples should be prepared and treated exactly as the samples undergoing the cleanup.

Hope the above information is helpful to you.

November 17, 1998

CALIFORNIA LABORATORIES APPROVED TO PERFORM PERCHLORATE ANALYSIS ON DRINKING WATER

Advanced Technology Laboratories
1510 E. 33rd St.
Signal Hill, CA 90807
(562) 989-4045

Aerojet Analytical Laboratories (**non-commercial lab?**)
Building 02030
P.O. Box 15847
Sacramento, CA 95852-1847
(916) 355-2209

Agricultural and Priority Pollutants (APPL), Inc.
4203 West Swift
Fresno, CA 93722
(209)-275-2175

Applied Physics & Chemistry Laboratory
13760 Magnolia Ave.
Chino, CA 91710
(909) 590-1828

E.S. Babcock and Sons, Inc.
6100 Quail Valley Court
Riverside, CA 92507
(909) 653-3351

California Laboratory Services
3249 Fitzgerald Road
Rancho Cordova, CA 95742
(916) 638-7301

City of Pasadena (**non-commercial lab**)
Water and Power Department
150 S. Los Robles Avenue, Suite 200
Pasadena, CA 91101
(626) 744-4411

Clinical Laboratory of San Bernardino, Inc.
P.O. Box 329
San Bernardino, CA 92402
(909) 825-7693

Columbia Analytical Services, Inc.
2059 Junction Avenue
San Jose, CA 95131
(408) 437-2400

County of Los Angeles (**non-commercial lab**)
Environmental Toxicology Laboratory
11012 Garfield Avenue, Bldg. B
South Gate, CA 90280
(562) 940-6778

Del Mar Analytical
2852 Alton Ave.
Irvine, CA 92606
(714) 261-1022

Metropolitan Water District of Southern California (**non-commercial lab**)
P.O. Box 54153
Los Angeles, CA 90054
(213) 217-6000

Montgomery Watson Laboratories
555 East Walnut Street
Pasadena, CA 91101
(818) 568-6400

Orange County Water District (Main Laboratory) (**non-commercial lab**)
P.O. Box 8300
Fountain Valley, CA 92728
(714) 378-3200

Santa Clara Valley Water District Laboratory (**non-commercial lab**)
5750 Almaden Expressway
San Jose, CA 95118-3686
(408) 265-2600

United Technologies Corporation (**non-commercial lab?**)
Chemical Systems Division
600 Metcalf Road
San Jose, CA 95138
(408) 776-4214

Weck Laboratories, Inc.
14859 East Clark Avenue
Industry, CA 91745
(818) 336-2139

AMERICAN PACIFIC CORPORATION

AMPAC

October 27, 1998

Ms. Brenda Pohlman
Remedial Action Program Supervisor
State of Nevada Department of Conservation and
Natural Resources
Division of Environmental Protection
Bureau of Corrective Actions
555 E. Washington, Suite 4300
Las Vegas, Nevada 89101

Subject: Sampling for Perchlorate in Selected Monitoring Wells near the Former Pepcon Site

Dear Ms. Pohlman:

This letter is intended to identify monitoring wells proposed to be sampled shortly for perchlorate, with results from a certified laboratory forwarded to NDEP, near what is referred to as the "western plume" identified in the May, 1998 report, "Hydrogeologic Investigation of Perchlorate in Ground Water at the Former Pepcon Plant, Henderson, Nevada." The following is a proposed list of monitoring wells:

MW-A	MW-AJ	—
MW-AL	MW-R	—
MW-L	MW-Q	—
MW-F2	MW-S	
MW-AE	MW-U	
MW-W	H-18	
MW-AH	H-12	—
— MW-J	MW-C	
MW-K	PL-641	—
— PL-645		

Best to Monitor.

H 12	* PL-635
* H 25	* PL-637
MW-J	PL-641
MW-R	PL-645
MW-Q	* PL-651
MW-AJ	* PL-653

* = NOT ON THEIR LIST

After receiving comments from you on these proposed wells, we will have them sampled for perchlorate in the near future.

Sincerely,

Jeff Gibson

JG/g

Post-it® Fax Note 7671	Date 11/2	# of pages ▶ 2
To Joe Donnelly	From Brenda Pohlman	
Co./Dept.	Co.	
Phone #	Phone #	
Fax #	Fax #	

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PHONE (702) 735-2200

486-2857 (FAX 28